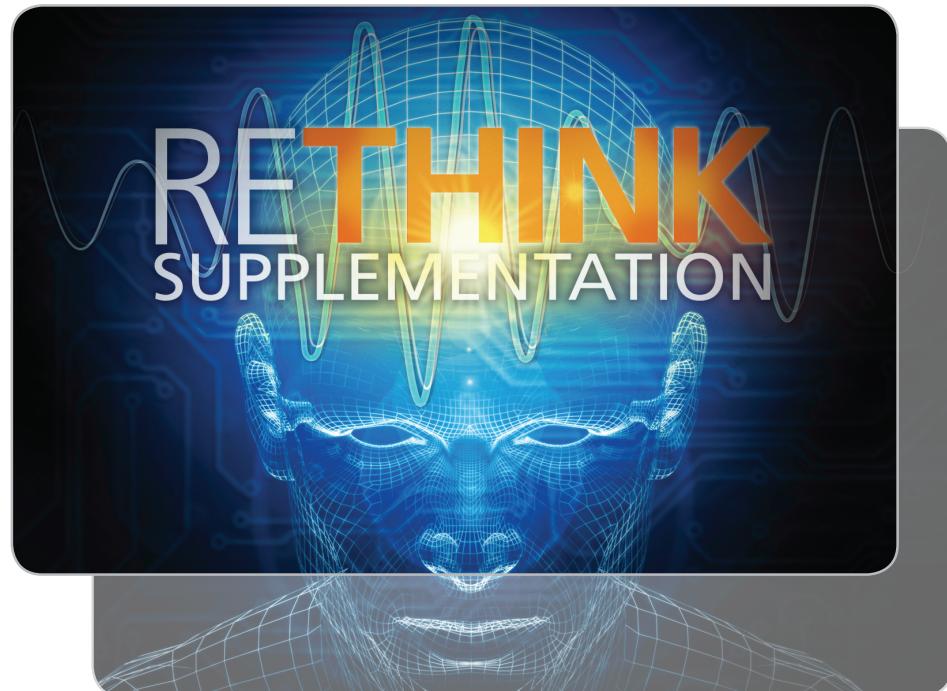


BD Resurge™ CD1–CD5, CD PAK
(without Glucose and L-Glutamine)



User Manual

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INTRODUCTION

Cell-based production of active pharmaceutical ingredients generally requires supplementation of cell culture media to promote cell growth and boost protein production. Every cell line can offer unique challenges for devising processes that deliver desired performance. One bioprocess challenge is the selection and optimization of a cell culture supplement or feed that will give desired titer, growth characteristics, and protein quality. Therefore, the availability of multiple chemically defined (CD) supplements offering a diverse performance profile can significantly improve process development timelines.

BD Biosciences – Advanced Bioprocessing has designed and formulated a diverse set of fully chemically defined (CD) and animal free (AF) cell culture supplements, BD Resurge™ CD1-CD5 supplements, without glucose and L-glutamine. This set of supplements can be used in batch and fed-batch processes to enhance

recombinant protein production of CHO cells while retaining suitable product quality. Optimal results with BD Resurge supplements can be attained by varying supplement concentrations and cell culture feeding regimen. These supplements have been tested on a variety of host CHO cells and in various culture systems including shaker flasks and bench-scale bioreactors. As a single supplement may not be ideal for enhancing performance across multiple cell lines conditions, a wider range of CD supplement formulations could be more effective for process development.



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PRODUCT INFORMATION

BD Resurge™ CD1-CD5 supplements are manufactured at the BD Biosciences – Advanced Bioprocessing animal-free and antibiotic-free (AF²™), state of the art facility. All the components are chemically defined with known molecular formulae

and structures and meet BD's stringent **Animal Origin-Free Specification**. These supplements are available as individual BD catalog numbers (Table 1) and as a kit – BD Resurge CD PAK (Table 2).

Table 1. Product information – BD Resurge CD1-CD5

Product Name	BD Catalog#	Qty	Unit Size
BD Resurge™ CD1	670011	100G	EACH
	670012	1KG	EACH
	670013	5KG	EACH
BD Resurge™ CD2	670015	100G	EACH
	670016	1KG	EACH
	670017	5KG	EACH
BD Resurge™ CD3	670018	100G	EACH
	670019	1KG	EACH
	670020	5KG	EACH
BD Resurge™ CD4	670021	100G	EACH
	670022	1KG	EACH
	670023	5KG	EACH
BD Resurge™ CD5	670024	100G	EACH
	670025	1KG	EACH
	670026	5KG	EACH

Table 2. Product information – BD Resurge CD PAK

Product Name	Qty	Unit Size	BD Catalog#
BD Resurge CD1	100G	EACH	Kit Cat# 670030
BD Resurge CD2	100G	EACH	
BD Resurge CD3	100G	EACH	
BD Resurge CD4	100G	EACH	
BD Resurge CD5	100G	EACH	

RECONSTITUTION PROCEDURE

Reconstitution at 40 g/L

BD Resurge™ CD1-CD5 supplements are supplied as powders and are recommended to be hydrated at 40g/L using the following instructions:

- Weigh 40 grams of BD Resurge CD supplement powder
- Fill a clean 1L beaker with approximately 900 mL room temperature (~25°C) water for injection (WFI) or equivalent
- Add the powder to the beaker and mix for a minimum of 30 minutes
- Once completely dissolved, bring volume to 1L with WFI or equivalent
- Measure and record pH and osmolality
- Filter sterilize the concentrated solution through a 0.2 μ m filter membrane and use as needed
- Store solution at 2-8°C protected from light. Use within six months of reconstitution or the expiration date of the original powder stock, whichever is earliest

Reconstitution at 100 g/L

More concentrated stock solutions of BD Resurge™ CD supplements can be prepared using the following instructions for making a 100g/L stock solution

- Weigh 100 grams of BD Resurge CD supplement powder
- Fill a clean 1L beaker with approximately 700 mL room temperature water for injection (WFI) or equivalent
- Add the powder to the beaker and mix until all the powder is in suspension
- Measure and record pH and osmolality
- Adjust the pH to between pH = 9.0 and pH = 10.0 using 5N or 6N NaOH
- Mix for a minimum of 30 minutes
- Once completely dissolved, adjust pH to pH = 8.0 \pm 0.2 or desired pH using 5N or 6N HCl
- Bring volume to 1L with WFI or equivalent
- Measure and record pH and osmolality

Note: BD Resurge reconstituted at 100 g/L may not meet specifications defined in Table 3.

- Filter sterilize the concentrated solution through a 0.2 μ m filter membrane and use as needed
- Store solution at 2-8°C protected from light. Use within one month of reconstitution or the expiration date of the original powder stock, whichever is earliest
- Continue to monitor for precipitation and color change

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SPECIFICATIONS OF RECONSTITUTED SUPPLEMENTS

Reconstituted BD Resurge™ CD supplements (at 40g/L) should have osmolality and pH as illustrated in Table 3 below.

Table 3. Osmolality and pH of BD Resurge CD1-CD5 at 40g/L BD Resurge

Product Name	Specification (mOsm/Kg)	Specification (pH)
BD Resurge Supplements CD 1	360 - 440	8.0 - 8.6
BD Resurge Supplements CD 2	360 - 440	8.0 - 8.6
BD Resurge Supplements CD 3	415 - 475	6.5 - 7.0
BD Resurge Supplements CD 4	400 - 460	8.0 - 8.6
BD Resurge Supplements CD 5	400 - 460	7.8 - 8.2

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STORAGE AND SHELF LIFE

BD Resurge™ CD1-CD5 supplements powder and reconstituted stock solutions should be stored at 2-8°C. Refer to the product label for the expiration date of each powder supplement when stored at 2-8°C. Stock solutions of BD Resurge CD supplements should be protected from light and should be used within six months of reconstitution or the expiration date of the original powder stock, whichever is earliest. Higher concentrations of BD Resurge CD supplements will have the same chemical stability but possibly different physical stability due to possibility of precipitation.

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TESTING PROCEDURES

6.1

Testing Procedure

BD Resurge™ CD1-CD5 supplements can be reconstituted and/or supplemented based on your specific culture or process requirements. The procedures in this manual are recommended as starting points.

When using the BD Resurge CD Supplement PAK for the first time, it is recommended that all five supplements be concurrently evaluated to determine the

supplement(s) that are most optimal for your system. For a batch culture process, an initial titration using each BD Resurge CD supplement is recommended. For this test, each of the supplement(s) can be used at a final supplemental concentration of 1g/L, 3g/L, and 6g/L on Day 0 or Day 2 of the culture. Glucose and glutamine should be maintained as per cell line requirements.

6.2

BD Resurge™ Titration – Batch Culture in shaker flask

Step 1 – Prepare shake flasks by adding an appropriate volume of reconstituted BD Resurge CD supplement (40 g/L) and basal medium to result in the final concentrations shown in Table 4.

Table 4. Experimental Set up for testing of a BD Resurge supplement

Shaker ID	Final BD Resurge Concentration (g/L)	Volume of Base Medium (mL)	Volume of BD Resurge (40 g/L) (mL)
BD Resurge supplement	1	48.75	1.25
BD Resurge supplement	3	46.25	3.75
BD Resurge supplement	6	42.54	7.46
Medium only (negative control)	0	50	0

Note: This table provides guidance for titration of one BD Resurge CD supplement, similar set ups can be used for all supplements; add appropriate positive control if needed. The titration data from BD Resurge supplements can then be evaluated and/or compared with the positive control to establish the ideal BD Resurge concentration.

Step 2 – Prepare the seeding culture according to your standard protocol. If cells are being cultured in a hydrolysate-containing medium, wash cells once in sterile PBS and pellet by centrifugation. Prepare seeding the cell suspension by resuspending the pellet into a base media (e.g. 3×10^7 cell/mL).

Step 3 – Inoculate shaker flasks with your usual seeding density (e.g. final seeding at 3×10^5 cell/mL).

Step 4 – On Days 0, 3, 5, 7, 10, and on the last day of cell culture, determine the viable cell density and percent viability.

Step 5 – Adjust glucose and glutamine levels as appropriate throughout the experiment.

Step 6 – Determine protein titer on various days of culture including the last the day to determine cumulative protein production.

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TESTING PROCEDURES (cont.)

6.3

BD Resurge™ CD1-CD5 supplements – Fed Batch Culture

For a fed batch process, BD Resurge CD Supplements can be used at a final supplemental concentration of 2-4g/L per day for multiple days, starting on Day 0 or Day 2 to mid-growth phase. The culture should be started at a reduced volume to accommodate the anticipated feeding volume. Glucose and glutamine should be maintained as per cell line requirements.

6.4

BD Resurge CD1-CD5 supplements – Bioreactor

Select two or three optimal concentrations of BD Resurge CD Supplements from the shaker flask batch culture study for the bioreactor batch study. Monitor and control glucose and glutamine levels as defined for the control process. Monitor process variables as well as metabolites, including lactate and ammonia, to assess cell culture performance. For fed-batch processes in bioreactors, we recommended testing a minimum of two feeding schemes that show promising results at the shaker flask level. Further optimization of the feeding scheme should be done based on metabolite levels and the bioreactor system.

Note: If using a cell line for the first time in a bioreactor system, we recommend optimizing the bioreactor parameters for the specific cell line and medium before supplementation with BD Resurge supplement(s).

COMPATIBILITY STUDIES

For troubleshooting any compatibility issues experienced while using BD Resurge™ supplements with a basal medium in cell culture processes, it is important to determine the effect

of the supplement on basal medium characteristics. A supplement would be considered compatible with the basal medium if the following characteristics are observed (Table 5):

Table 5. Required Compatibility Characteristics of BD Resurge™ Media Supplements

Characteristic	Requirement	Notes
Chemical Interaction	No color change due to supplement addition to cell culture medium	Color change due to addition of supplement may suggest presence of chemical reactions
Osmolality	Should not be significant to affect osmolality of basal cell culture medium	Use base medium osmolality and the product information above (Table 3) to determine suitability
pH	Should not be significant to affect the pH of cell culture medium	Use base medium pH and the product information above (Table 4) to determine suitability
Precipitation	No precipitate formation due to supplement addition to cell culture medium	Observed visually after addition of the supplement to the basal medium. Precipitation would indicate solubility issues or chemical reaction

8.1 Representative Data

BD Resurge™ CD1-CD5 supplements can be used in batch and fed batch modes in cell culture processes. Figure 1(A-E) demonstrates the performance of BD Resurge CD1-CD5 in batch and fed batch mode in shaker flasks.

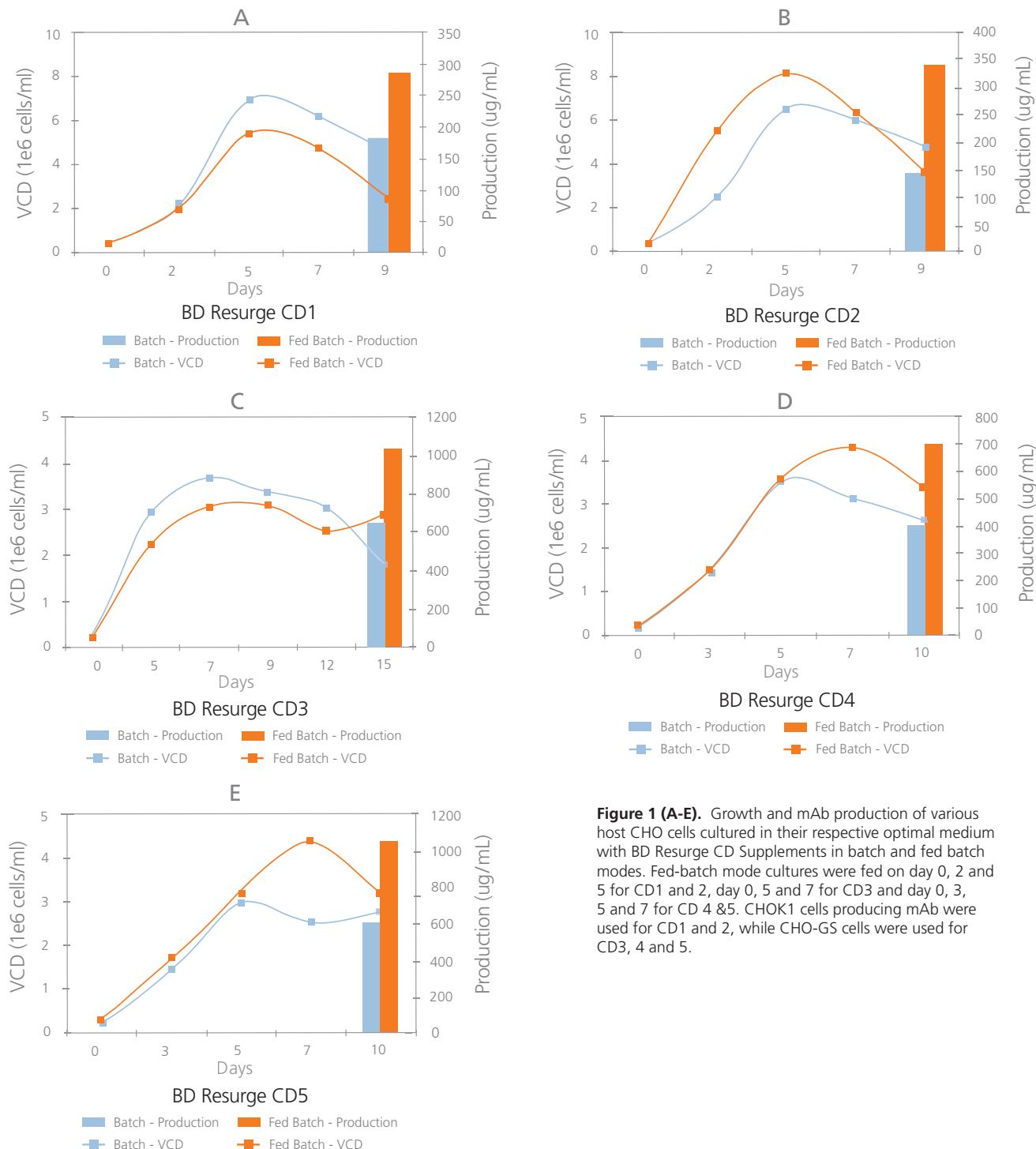


Figure 1 (A-E). Growth and mAb production of various host CHO cells cultured in their respective optimal medium with BD Resurge CD Supplements in batch and fed batch modes. Fed-batch mode cultures were fed on day 0, 2 and 5 for CD1 and 2, day 0, 5 and 7 for CD3 and day 0, 3, 5 and 7 for CD 4 &5. CHOK1 cells producing mAb were used for CD1 and 2, while CHO-GS cells were used for CD3, 4 and 5.

8.2 Performance Data

Performance of BD Resurge™ CD supplements is scalable to bioreactors. BD Resurge CD supplements produced a 2-3-fold increase in protein production over media control in bench top bioreactors in batch or fed batch mode (Figures 2A-B).

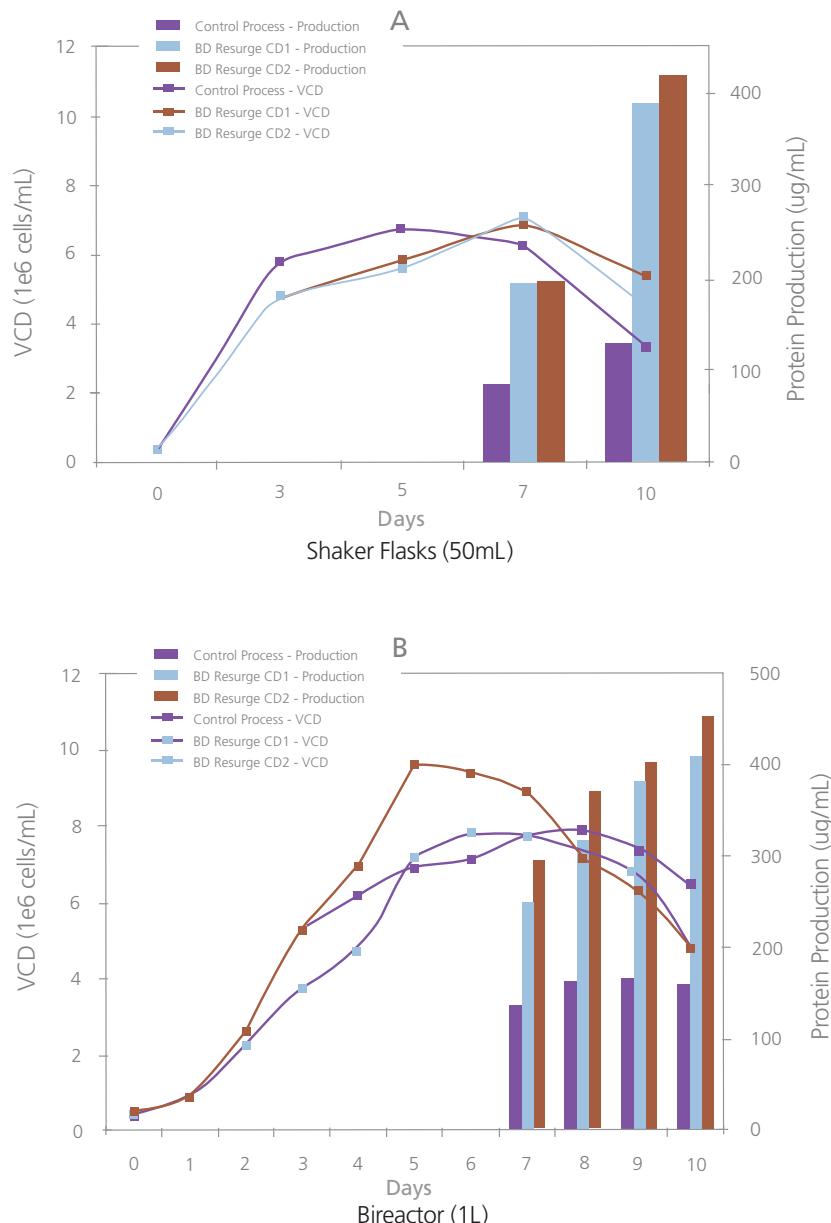


Figure 2(A-B). Representative data showing growth and production of CHO K1 cells fed with BD Resurge CD1 and CD2 in shaker flask (50 mL culture) (Fig 2A) and stir tank bioreactor (1L) (Fig 2B). Study was performed in fed batch mode using BD Resurge CD1 and CD2 on Day 3 and 5 of culture in addition to control process. Data demonstrates the scalability of CD1 and CD2 from shaker flask to bioreactor.

SUMMARY AND RECOMMENDATIONS

- BD Resurge™ supplements CD1-CD5 are animal free, protein free, and chemically defined, and can be used in batch and fed batch mode in cell culture processes. Supplementation with BD Resurge CD supplements is scalable and has been demonstrated to perform in shake flasks, bioreactors and other culture systems (data not shown).
- Although the development of the BD Resurge supplements focused on CHO cell lines expressing monoclonal antibodies and recombinant proteins, their application can certainly be investigated on other host cell lines.
- This manual provides guideline for usage of BD Resurge supplements CD1-CD5; however, each cell line would need further optimization for optimal performance. For further questions contact BD Technical services at 1-800-638-8663.

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TROUBLESHOOTING

Table 6. Trouble Shooting Guidelines

Problem	Cause	Solution
BD Resurge™ supplements are either not dissolving or taking excessively long time to completely dissolve	Low vortex due to slow mixing speed or wrong reconstitution vessel	<ul style="list-style-type: none"> Use a beaker to reconstitute small amounts of BD Resurge supplements Increase the mixing speed to maximize the vortex
	Temperature of the water or room is unusually low	<ul style="list-style-type: none"> Reconstitute with room temperature (~ 25°C) water in an environment controlled at ~ 25°C
Medium is cloudy or a precipitate is observed following addition of BD Resurge supplements	Possible reaction between components resulting in undissolved products. Concentration of some components above solubility limits.	<ul style="list-style-type: none"> Perform compatibility test to identify the correct concentration of BD Resurge supplements to use Contact BD Technical Services for trouble shooting unwanted chemical reactions
	Possible contamination	<ul style="list-style-type: none"> Identify contaminant and root cause of contamination Retest
Color of medium containing BD Resurge supplements changes during storage	Possible reaction between components or degradation of the components	<ul style="list-style-type: none"> Contact BD Technical Services for trouble shooting unwanted chemical reactions
High osmolality and pH after addition to medium	High concentration of BD Resurge used	<ul style="list-style-type: none"> Perform compatibility test to identify the optimum concentration of BD Resurge to use for your application
Low cell growth and low cell viability	High levels of BD Resurge	<ul style="list-style-type: none"> Perform BD Resurge supplements titration study to determine appropriate concentration range Re-check calculations for amount of BD Resurge added
	High osmolality due to use of other additives like amino acid feeds, hydrolysates feeds etc.	<ul style="list-style-type: none"> Perform compatibility test to identify the optimum concentration of BD Resurge to use for your application. Reduce other supplementation levels
	Over supplementation if using other feed additives (amino acids, hydrolysates etc) together with BD Resurge	<ul style="list-style-type: none"> Try alternate feeding plan with reduced amount of other feeds (amino acids, hydrolysates etc.)
	Timing of supplementation requires further optimization	<ul style="list-style-type: none"> Try various days of cell culture supplementation, including Day 0 supplementation, to determine optimal feeding conditions

10 TROUBLESHOOTING

Table 6. Trouble Shooting Guidelines

Problem	Cause	Solution
Low protein production	Low cell growth and low cell viability	<ul style="list-style-type: none">• See “Low cell growth and low cell viability” section
	Not sampling on the day of peak protein production	<ul style="list-style-type: none">• Sample multiple time points until cell viability decreases below 50% in order to determine time of peak protein production
	Timing of supplementation requires further optimization	<ul style="list-style-type: none">• Try various days of cell culture supplementation, including Day 0 supplementation, to determine optimal feeding conditions• Try alternate feeding pattern• Shift temperature to reduce cell growth• Use cells from log phase of culture (when cells are in their growth phase) when seeding the cells• Use high seeding density
Rapid cell growth and low protein production	Energy shifted towards cell growth	

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